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Retention of BHA in Atlantic salmon (*Salmo salar*)

Introduction

Butylated hydroxyanisol (BHA) is a phenolic antioxidant that is used as a food additive, particularly in fats and oils, and it is also authorized as a feed additive in the EU for all animal species with a maximum concentration of 150 mg/kg feed (alone or in combination with ethoxyquin and/or butylated hydroxytoluene). The safety of BHA has been evaluated several times since 1961 by the *Joint FAO/WHO Expert Committee on Food Additives*, and the latest acceptable daily intake (ADI) was set at 0 – 0.5 mg/kg bw (JECFA, 1989). Information on the BHA levels in food of animal origin is scarce, as is literature on the transfer of BHA from feed to animal products. Consequently a 14 week feeding trial has been conducted in order to determine the retention of BHA in Atlantic salmon (*Salmo salar*) fillets. Four different concentrations of BHA in feed were tested, and fish were sampled three times during the three month exposure period in order to examine dose response as well as the effect of exposure duration. In order to assess the elimination of BHA from Atlantic salmon, fillet samples were analysed after a two week starvation period which is representative of that mandatory for commercially farmed salmon. Effects of dietary BHA exposure on fish health were also monitored during the study.

Materials and methods

Feeds based on fish meal and fish oil with four different BHA concentrations were produced by EWOS. The analyzed content of ash, dry matter, protein, fat and energy in the feeds was 7.5 - 7.6 %, 92.3 - 92.7%, 40.9 - 42.7%, 27.3 - 28.9% and 24.1 – 24.5 kJ/g, respectively. BHA was dissolved in fish oil, contributing 1% to the total feed, before it was added through coating. The analyzed BHA concentration was 0 (A), 48.5 (B), 92.5 (C) and 225 (D) mg BHA kg^{-1} feed. The highest dose exceeded the maximum permitted concentration in the EU (150 mg BHA kg^{-1}) to assess the resulting deposition in the fillet.

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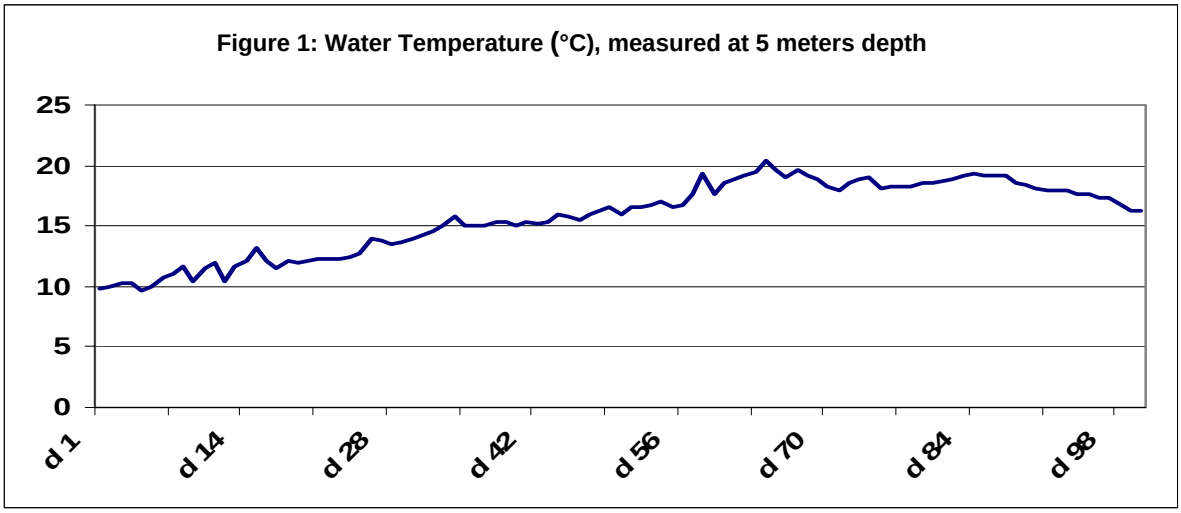
From a batch of approximately 3000 Atlantic salmon (*Salmo salar*), kept in one sea net pen at the research site (Fiskeriforskning, Austevoll, Norway), 12 randomly chosen fish were sacrificed as the day 0 control group. Six hundred fish weighing between 750 -800 g were taken from the holding net pen and randomly distributed among 12 net pens (triplicate net pens were used for each of the four treatments), so that each of the net pens contained 50 fish with a total bio mass of 38.4 – 39.1 kg. Feeding with experimental diets began one day later and the fish were fed to satiation once a day with a manually operated feeder, the feed intake could only be roughly estimated. Although the fish were kept in smaller net pens (5 x 5 x 5 meter) and at a lower density, the experimental conditions were comparable to commercial salmon production.

The seawater temperature rose considerably during the study which lasted from the 1st of June to 7th of September 2006. Figure 1 shows the water temperature taken at 5 meters depth. Due to sea lice (*Lepeophtheirus salmonis*) infestation it was necessary to treat the fish twice (in week 7 and 11) with a deltamethrin bath (Alphamax™).

Figure 1: Water Temperature (°C), measured at 5 meters depth



Fish were sampled after 4, 8, and 12 weeks dietary exposure, and after a two week starvation period, which is representative for commercial salmon farming in Norway. At each of the sampling points three fish were sampled per net pen (nine fish per treatment). An additional three fish were sampled but not sacrificed at each sampling point in order to measure body weight and length.

Table 1: Effect of graded levels of BHA in feed on weight and condition factor (mean and standard deviation) of Atlantic salmon (*Salmo salar*)	Table 2: Effect of graded levels of BHA in feed on liver weight and liver somatic index (mean and standard deviation) of Atlantic salmon (*Salmo salar*)

The fish were anaesthetized, weighed, length was measured, and after blood had been taken from the caudal vein, they were killed by a blow to the head. In the day zero control group, the measurements were weight, length and BHA concentration in the fillet. On the other sampling days liver tissue and blood were also sampled. Sodium (Na), potassium (K), chloride (Cl) and glucose concentrations in the blood were determined immediately. Serum, liver tissue and intestinal epithelial cells were frozen in liquid nitrogen for subsequent measurements of health parameters. The fillet samples were homogenized on site, and stored on dry ice until arrival at the institute, where they were stored at -80°C until analyses (HPLC). Body measurements were used to compute condition factor (CF = 100 * bw / length^3) as well as liver somatic index (LSI = liver weight *100 / body weight).

Results

The approximate total feed consumption during the study was 19.5 – 23.2 kg in the individual net pens, and 57.2 – 64.6 kg per treatment, the highest feed consumption was recorded for the highest dose group (D), followed by A, B and C. The mortality was relatively high (2 – 13 animals per net pen), however the total number of mortalities per group (A: 31; B: 18; C: 31; D: 17) does not indicate that this was treatment related.

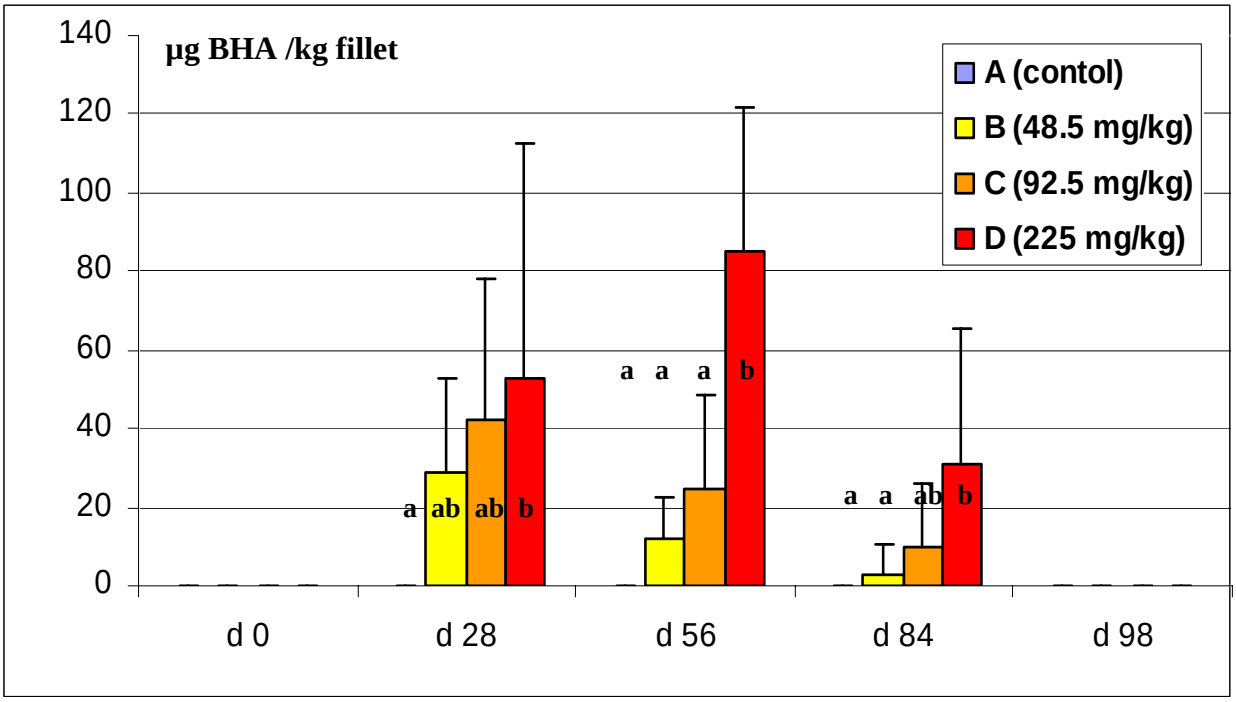
Length (data not shown), weight and CF (Table 1) were not significantly different among the treatments at any time point. The fasting period reduced weight but not length, consequently CF was slightly altered. Liver weight and LSI (Table 2) did not differ among the groups. Liver weight increased only until the 8th week thereafter it remained stable (12th week), followed by a slight reduction in the fasting period. LSI changed accordingly. Blood ions (Na, K), pH and glucose did not differ significantly among the groups at ant time point, and chloride (Cl) was always above (>140 mmol/l) the detection range (data not shown).

	A contol	B 48.5 mg/kg	C 92.5 mg/kg	D 225 mg/kg		A contol	B 48.5 mg/kg	C 92.5 mg/kg	D 225 mg/kg
Weight (g)					**Liver Weight (g)**				
D 0 (n = 12)	712.1 *157.9*				D 0 (n = 9)	6.7 *2.0*			
D 28 (n = 9)	845.7 *180.8*	901.6 *149.3*	894.0 *122.2*	803.4 *158.7*	D 28 (n = 9)	8.2 *1.3*	9.7 *2.9*	9.6 *1.9*	7.6 *1.8*
D 56 (n = 9)	1027.8 *175.3*	1061.3 *134.1*	1048.0 *232.1*	1085.3 *189.8*	D 56 (n = 9)	10.5 *3.4*	11.5 *1.86*	10.9 *2.9*	11.6 *2.2*
D 84 (n = 9)	1277.4 *225.7*	1185.8 *231.2*	1166.6 *111.0*	1151.9 *203.0*	D 84 (n = 9)	10.1 *1.5*	10.0 *2.0*	11.0 *1.6*	9.7 *2.5*
D 98 (n = 12)	1043.6 *267.3*	1148.1 *317.3*	1117.7 *302.8*	1065.9 *245.0*	D 98 (n = 9)	6.9 *1.5*	8.8 *1.4*	9.3 *3.1*	8.2 *2.9*
Condition factor					**Liver Somatic Index**				
D 0 (n = 12)	1.11 *0.09*				D 0 (n = 9)	0.93 *0.16*			
D 28 (n = 9)	1.13 *0.09*	1.19 *0.09*	1.24 *0.15*	1.14 *0.07*	D 28 (n = 9)	0.99 *0.21*	1.06 *0.18*	1.09 *0.26*	0.95 *0.11*
D 56 (n = 9)	1.19 *0.11*	1.19 *0.05*	1.19 *0.08*	1.19 *0.07*	D 56 (n = 9)	1.01 *0.23*	1.10 *0.19*	0.99 *0.11*	1.07 *0.16*
D 84 (n = 9)	1.23 *0.08*	1.15 *0.07*	1.26 *0.06*	1.18 *0.12*	D 84 (n = 9)	0.73 *0.28*	0.86 *0.14*	0.95 *0.15*	0.83 *0.11*
D 98 (n = 12)	1.08 *0.10*	1.09 *0.08*	1.07 *0.18*	1.09 *0.08*	D 98 (n = 9)	0.69 *0.11*	0.75 *0.12*	0.76 *0.11*	0.70 *0.17*

BHA was not detectable in the fish fillets at the start of the experiment (day 0 control group, Table 3). Similarly BHA was not detected in any of the control fish during the study. The BHA concentrations in fish fillets from the three BHA supplemented groups were dose dependent, however but no time response was observed. After the two week elimination period BHA was under the detection limit (< 7 µg/kg) in fish fillets from all treatments.

Figure 2. Mean (and standard deviation) retention of BHA in fillets of Atlantic salmon (Salmo salar) fed graded levels of BHA in their diet for 84 days followed by 14 days

starvation.



Discussion

The feed intake reduced during the experiment, and, accordingly, growth performance was lower than expected. This may be explained by the high seawater temperature, furthermore, the sea lice infestation, boosted by the temperature, and the therapy is also likely to have negatively influenced feed intake. Similarly both parasite infestation and high seawater are likely to have caused the high mortality seen in the present study. Although the experimental conditions were far from optimal, growth and body parameters as well as the general health (blood chemistry) parameters, analyzed, do not indicate that exposure of Atlantic salmon to BHA in the feed had any negative effects on fish health.

The BHA content in the fillets was generally low, however there was considerable variation within treatments. The group means appeared to be reduced by animals with no detectable BHA in the fillet, observed at all time points and in all groups. The relatively low feed intake is one possible explanation for this. However, it has been shown that BHA is rapidly eliminated in rats (Ansari and Hendrix, 1985). After oral administration of a single dose C^{14} labeled BHA isomer (3-*t*-[methyl-^{14}C]butyl-4-

hydroxyanisole) radioactivity reached a peak within 6 hours in several organs and after 48 hours almost all radioactivity was eliminated. Supposing that the elimination time for BHA is similar in rats and salmon, and considering that there was a time period of 20 to 26 hours between last feeding and sampling the low concentrations found in the present study may be explained by partial elimination.

The levels of BHA in salmon fillets were dose dependent throughout the feeding period. However, the concentrations did not increase during the study indicating that accumulation is prevented by rapid elimination/catabolism. This also explains why BHA was not detectable (< 7 µg/kg) in fish fillets following the two week elimination period.

There is limited information on the metabolic fate as well as on pharmacokinetics and -dynamics of BHA in fish. The current study focused on the retention of BHA in muscle tissue since only the fillets of Atlantic salmon are of relevance to the consumer. Although there was carry-over of BHA from the feed to the fillet (the highest measured concentration was 183 µg/kg), BHA was below the limit of detection (<7 µg/kg) in salmon fillets following 14 days starvation, which is mandatory in salmon production in Norway. Consequently the maximum residue level of 50 µg BHA/kg proposed by Japan does not appear to cause any limitations for the export of Norwegian farmed Atlantic salmon.

References

Ansari, G.A.S. and Hendrix,P.Y., 1985. Tissue distribution and pharmacokinetics of 3-t-[Methyl-^{14}C]butyl-4-hydroxyanisole in rats. Drug Metabolism and Disposition 13 (5), 535-541.

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